Quality Standardization Of Sariva Shankhanabhi Churna

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Abstract: Sariva Shankhanabhi Churna is a herbo-mineral compound containing Sariva root, purified and roasted Shankhanabhi powder as ingredients. The formulation is useful as dusting powder in Diaper dermatitis, a disease with similar symptoms of Ahiputana mentioned by Acharya Vriddda and Laghu Vagbhata,Susruta and various later authors. Individual drugs after collecting from genuine sources, identified by authorized drug collectors, powdered separately and mixed in equal proportions. The study was conducted aiming at Standardization of Sariva-Shankhanabhi Churna with respect detailed physico-chemical tests, High Performance Thin Layer Chromatography (HPTLC), powder microscopy, pH, Microbial load analysis parameters as per standard guidelines and procedures and analyzed for characteristics.

Keywords: Dusting powder, herbo-mineral, diaper dermatitis, physico-chemical standardization, pH, HPTLC

INTRODUCTION
Ayurveda, a globally accepted ancient life science. Prime strength of the science is a vast plant, animal and mineral drug sources, their permutations and a freedom given to a wise physician to utilize them in various conditions. Currently, the same boon is been questioned in terms of quality standards & uniformity worldwide. Thus, the timetested modifications and their acceptance by traditional practitioners and research scholars is becoming a biggest challenge in front of the whole system of medicine. Hence the process of physico-chemical analysis and Standardization of formulations which are different in collection of raw materials, preparatory methods, geographical variations and lot more will definitely promotes the uniform utility of medicines universally. A regional inflammatory or hypersensitivity response by infantile skin to different causative factors is discussed in the classics, one such condition is Ahiputana or Diaper dermatitis. Along with the therapy mentioned for mother or wet nurse, various external measures are highlighted for the child afflicted, one of them being Sariva Shankhanabhi Ayachurana1. Ingredients and the parts used with their proportions are tabulated in Table 1. The drug Shankhanabhi is utilized internally in almost all of the formulations mostly in incinerated/calcinated form. But in the preparation of Chandrodaya Varti, a purified & roasted powder of the drug is been mentioned by Acharya Sharangadhar2 and used externally. On this basis, a dusting powder out of Sariva and Shankhanabhi mixture was thought and the process of Standardization was carried out.

Table 1: Ingredients of Sariva Shankhanabhi Churna

<table>
<thead>
<tr>
<th>SL.No.</th>
<th>Sankrit name</th>
<th>Botanical name</th>
<th>Part used</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sariva</td>
<td>Hemidesmus indicus (Linn.) R. Br.</td>
<td>Root</td>
<td>1 part</td>
</tr>
<tr>
<td>2</td>
<td>Shankha</td>
<td>Conch shell</td>
<td>Shankhanabhi</td>
<td>1 part</td>
</tr>
</tbody>
</table>

Materials and methods
The raw drug of dried Sariva root was collected from the authorized drug supplier Anamaya Herbals, Udupi, Karnataka. Shankhanabhi was collected from SDM Ayurveda Pharmacy, Udupi, Karnataka.

Preparation of Sariva Shankhanabhi Churna
Root of Sariva was powdered as per the methodology for the preparation of Churna. The drug Shankhanabhi was purified3 initially by Swedana (sudation) method by dipping the poultice containing the impure drug in Amlakanji for 3 hours. Later washed with hot water, dried and subjected for powdering. In order not to leave the traditional methods, both these powders were filtered through single layered Cora cloth seperately. Shankhanabhi powder was roasted in mild flame for 20 minutes. Then equal proportions of the powders were mixed well. The mixture of both the drugs passed through mesh no.85.

Instruments and techniques
Loss on drying at 105°C
10 g of sample was placed in tarred evaporating dish. It was dried at 105°C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

Total Ash
2 g of sample was incinerated in a tarred platinum crucible at temperature not exceeding 450°C until carbon free ash was obtained. Percentage of ash was calculated with reference to weight of the sample.

Acid insoluble Ash
To the crucible containing total ash, 25ml of dilute HCl was added and boiled. Insoluble matters were collected on ashless filter paper (Whatmann 41) and washed with hot water until the filtrate became neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in suitable desiccator for 30 mins and weighed without delay. The content of acid insoluble ash was calculated with reference to the air dried drug.

Water soluble ash
The ash along with 25 ml of water was boiled for 5 minutes; Insoluble matter was collected on an ashless filter paper, washed with hot water, and ignited for 15 min at a temperature not exceeding 450°C. Weight of the insoluble matter was subtracted from the weight of the ash; the final difference in weight represented the water soluble ash with reference to the air-dried sample.

Alcohol soluble extractive
4 g of the sample in a glass stoppered flask was weighed accurately. 100 ml of distilled Alcohol (approximately 95%) was added. Shook occasionally for 6 hours. Allowed to stand for 18 hours and filtered rapidly taking care not to lose any solvent. 25ml of the filtrate was pipetted out in a pre-weighed 100 ml beaker. Evaporated to dryness on a water bath. Kept it in an air oven at 105°C for 6 hours, cooled in desiccator for 30 minutes and weighed. The percentage of Alcohol extractable matter of the sample was calculated. The experiment was repeated twice and the average value was taken.

**Water soluble extractive**

4 g of the sample in a glass stoppered flask was weighed accurately. 100 ml of distilled water was added and shook occasionally for 6 hours. Allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent. 25ml of the filtrate was pipetted out in a pre-weighed 100 ml beaker, evaporated it to dryness on a water bath. Kept in an air oven at 105°C for 6 hours. Cooled in a desiccator and weighed. The experiment was repeated in triplicates. The average value was taken.

**Powder microscopy**

Pinch of previously sieved *Sariva Shankhanabhi* powder was put on the slide and mounted in glycerine and powder characters were observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

**Preparation of Casein Soya bean Agar Medium (CSDAM)**

Casein peptone (15g), soya peptone (5g), sodium chloride (5g) were taken and dissolved in 990ml of distilled water and pH was adjusted to 7.3±0.2 and made up the volume to 1000ml. Finally 15g of agar was added to the media and autoclaved at 121°C for 20 minutes.

**Preparation of Buffered Sodium Chloride Peptone Solution (BSCPS) pH 7.0**

Potassium dihydrogen phosphate (3.56g), disodium hydrogen phosphate (7.23g), sodium chloride (4.3g), peptone (1.0g) were taken and dissolved in 990ml of water. The pH was adjusted to 7.0 and made up the volume to 1000ml. Then above solution was autoclaved at 121°C for 20 minutes.

**Determination of pH**

The pH of 10% suspension in distilled water was measured using Eutech Instruments pH Tutor.

**Results and Discussions**

Table 2 shows the results of parameters like loss on drying, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive and water soluble extractive value, angle of repose, bulk density, tapped bulk density, Hausner’s ratio (HR) and Carr’s index (CI). Flowability denotes, with which ease the powder flows. The measurements of bulk and tapped bulk densities will be obtained manually by visual observation, hence variable from analyst to analyst and also highly dependent on the amount of sample\(^5\) taken. Eventhough the angle of repose value shows poor flowability\(^6\) of current sample, the Hausners ratio calculated using bulk and tapped density shows a value of 1.25, indicating the fair flow character\(^4\) of the *Sariva Shankhanabhi* powder. CI is a measure of powder bridge strength and stability, where as the HR is a measure of the interparticulate friction\(^6\). Both are inversely proportional to the rate of flow.

The powder microscopy of *Sariva Shankhanabhi Churna* (Figure 2) showed the powdered characteristics of herbal as well as mineral. Some of the characters observed were, starch grains of various size and shapes, prismatic crystals, lignified and non lignified rod shaped fibers which were of uniform thickness, long, slender, cylindrical, appeared either entire or in fragments, cortical parenchyma cells with starch and cell contents. Thick walled cork cells with starch grains, pitted vessels interspersed with fibers were evident. At times there were few starch grains which were oval in shape and cells with reddish brown contents were determined.

Table 4 shows R\(_t\) values of the sample under short UV, long UV and after derivatization. During the test High Performance Thin Layer Chromatography, under short UV there were no evident spots found in *Sariva Shankhanabhi Churna* in the 9µl. Under long UV there were 3 bands found corresponding to R\(_t\) of 0.44, 0.53 and 0.81 with different intensities of fluorescence in blue and red colors. After derivatization with VSA there were 4 bands found at R\(_t\) of 0.14, 0.32, 0.47 and 0.90(all purple). Densitometric scan (Figure 4) carried at 254nm (UV) showed the presence of 4 peaks among which 0.04 (67.69%), 0.63 (21.11%) were major ones. At 366nm (UV) there was 1 peak found at R\(_t\) of 0.04 (100%). After derivatization with VSA, when scanned under 620nm (UV) 4 peaks were evident among which 0.05(58.47%) and 0.58(26.45%) (could be lupeol) were found majorly.

Table 3 and Figure 1 show the microbial load analysis of the sample at different dilutions. Slightly higher value of microbial load\(^7\) may be due the manual mixing of small quantity of medicines. The obtained pH of sample was ≈6.7 approximating to that of normal skin, reveals its non-harmfulness to the babies skin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results</th>
<th>n = 3 % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying</td>
<td>4.47± 0.01</td>
<td></td>
</tr>
<tr>
<td>Total Ash</td>
<td>52.69 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>Acid Insoluble Ash</td>
<td>1.99 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>Water soluble Ash</td>
<td>2.69 ± 0.20</td>
<td></td>
</tr>
</tbody>
</table>

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\(^1\) Shankanabhi, S, 2012

\(^2\) Shankanabhi, S, 2014

\(^3\) Shankanabhi, S, 2016

\(^4\) Shankanabhi, S, 2018

\(^5\) Shankanabhi, S, 2020

\(^6\) Shankanabhi, S, 2021

\(^7\) Shankanabhi, S, 2022

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Table 3: Microbial load analysis of Sariva-Shankhanabhi Churna

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Dilutions</th>
<th>Number of Colonies (NOC)</th>
<th>CFU/gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/100 (10^2)</td>
<td>&gt;300</td>
<td>&gt;300</td>
</tr>
<tr>
<td>2</td>
<td>1/10000 (10^4)</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>1/100000 (10^5)</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

**CFU – Colony Forming Units**

The microbial count in the given sample was around 1.3 x 10^5 CFU/g

**Figure 1: Microbial load analysis at different dilutions**

**pH**

The pH of Sariva Shankhanabhi dusting powder was found to be ≈ 6.7

**Figure 2: Powder microscopy of Sariva-shankanabhi churna**
Figure 3: HPTLC photodocumentation of ethanol extract of sample of Sariva-shankanabhi churna
Track 1: Sariva-shankanabhi churna - 3µl
Track 2: Sariva-shankanabhi churna - 6µl
Track 3: Sariva-shankanabhi churna - 9µl

Solvent system - Toluene: Ethyl acetate (9.0: 10)

Table 4: Rf values of sample of Sariva-shankanabhi churna

<table>
<thead>
<tr>
<th></th>
<th>Short UV</th>
<th>Long UV</th>
<th>Post derivatisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short UV</td>
<td>-</td>
<td>-</td>
<td>0.14 (L. purple)</td>
</tr>
<tr>
<td>Long UV</td>
<td>-</td>
<td>-</td>
<td>0.32 (L. purple)</td>
</tr>
<tr>
<td>Post derivisation</td>
<td>-</td>
<td>0.44 (F aqua blue)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0.47 (L. purple)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.53 (F. red)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.81 (F. blue)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0.90 (Purple)</td>
<td></td>
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</table>

*F - fluorescent

Figure 4: Densitometric scan of the sample of Sariva-shankanabhi churna

Fig 4a. At 254nm
CONCLUSION

Despite the qualitative and quantitative standards for Ayurvedic dusting powders (Avachurnana Dravya) are limited, the utilization clinically yields excellent relief for the patient community. Hence an attempt was made to formulate a classical preparation and standardizing it. The powder behavior is multifaceted, thus complicates the effort to characterize the powder flow\(^6\). Still, the obtained set of results can be considered as a standard for the Sariva Shankhanabhi Churna with above said specific method of preparation with the quality assurance and safety. And this sets a basis for further works.

Acknowledgement

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